VRON-0200 alone and in combination with investigational antivirals for participants with chronic hepatitis B virus infection receiving stable nucleos(t)ide therapy: a phase 1b, randomised, open-label, multicentre trial

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Summary

Background Functional cure treatments for hepatitis B virus (HBV) have been restricted by their inability to restore anti-HBV immunity, often leading to viral rebound after treatment. VRON-0200 is a novel immunotherapy for functional cure of HBV that contains a checkpoint modifier together with HBV core and polymerase (pol) antigens—but not surface antigens—to enhance, broaden, and prolong T-cell responses with the aim to prevent after treatment viral rebound and improve functional cure response rates. We report the safety, tolerability, immunogenicity, and antiviral efficacy of VRON-0200 alone and in combination with investigational antivirals in participants with chronic HBV infection receiving nucleos(t)ide therapy.

Methods This phase 1b, randomised, open-label, multicentre trial included participants with chronic HBV infection aged between 18 and 55 years who had normal liver function, were receiving stable nucleos(t)ide therapy, and had non-detectable HBV DNA for 12 months or more. Participants with baseline HBsAg of less than 500 IU/mL were randomised to receive an intramuscular VRON-0200 prime alone on day 1 or with a VRON-0200 boost on day 91. In group 1, participants were randomised to receive either a low prime dose (1×10^{10} viral particles) of chimpanzee adenovirus serotype 7 (AdC7; group 1a) or chimpanzee adenovirus serotype 6 (AdC6; group 1b) by intramuscular injection, with group 1a participants receiving an AdC6 boost at day 91. In group 2, participants were randomised to receive either a high prime dose (5×10^{10} viral particles) of AdC7 (group 2a) or AdC6 (group 2b) by intramuscular injection, with group 2a participants receiving an AdC6 boost at day 91. In group 3, participants with baseline HBsAg of less than 1000 IU/mL were randomised to receive an intramuscular VRON-0200 prime, followed by six monthly doses of elebsiran plus tobevibart starting on day 28, alone, or with a VRON-0200 boost on day 196. The primary endpoint was to evaluate the safety and tolerability of VRON-0200, with additional immunological (interferon-γ [IFN-γ] enzyme-linked immunosorbent spot [ELISpot]) and virological assessments (HBsAg and HBV DNA) performed. IFN-γ ELISpot responses were assessed using a one-sided paired t-test (day 28 *vs* baseline), whereas a two-sided paired t-test was used to compare responses between day 154 and day 91 in participants receiving either the prime alone or prime–boost regimen. This study was registered at ClinicalTrials.gov (NCT06070051) and is active and fully enrolled.

Findings Between Oct 26, 2023, and March 17, 2026, 35 participants were enrolled (groups 1 and 2, n=27; group 3, n=8): 28 (80%) were male, 30 (86%) were Asian, and 34 (97%) were HBeAg negative. The median participant age was 46 years (range 37–55). Median baseline HBsAg was 179 IU/mL (range 16–623) in group 1, 149 IU/mL (10–563) in group 2, and 469 IU/mL (12–1169) in group 3. VRON-0200 was well tolerated, with 38 treatment-related adverse events reported (four of grade 2, 34 of grade 1); no treatment-related serious adverse events, discontinuations, or clinical laboratory abnormalities, including liver function test results, were reported. In groups 1 and 2, at day 360, 23 (85%) of 27 participants had sustained or continued HBsAg declines (12 [52%] of 23 had reductions of >50%) and four (15%) participants had declines of 1 $\log_{10}$ IU/mL or more from baseline. 12 participants had long-term follow-up to 846 days after VRON-0200 prime dosing; 11 (92%) of 12 participants had continued HBsAg declines, with seven (58%) having HBsAg concentrations of less than 10 IU/mL, four (33%) having less than 0·5 IU/mL, and two (17%) having less than 0·05 IU/mL (lower limit of detection [LLOD]). A boost dose was not observed to improve HBsAg declines. At day 28, IFN-γ ELISpot responses significantly increased 3·2 fold from baseline (p=0·007); however, these responses were not observed to be associated with HBsAg declines. In group 3, the addition of investigational antivirals resulted in rapid and profound HBsAg declines within 7 days (all seven [100%] participants treated with elebsiran plus tobevibart had HBsAg concentrations <2 IU/mL; one [14%] had HBsAg concentrations below the LLOD), regardless of baseline HBsAg concentrations. At day 196 (28 days after the last antiviral dose), all participants had HBsAg concentrations below 0·5 IU/mL (three [43%] of seven participants had HBsAg concentrations below the LLOD), and at day 259 (91 days after the last antiviral

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dose), six (86%) of seven participants had HBsAg concentrations that remained below 0·5 IU/mL and one (14%) had HBsAg concentration that remained below the LLOD.

Interpretation VRON-0200 alone or with investigational antiviral agents had no observed safety concerns and was well tolerated. Despite not targeting HBsAg, a single VRON-0200 dose induced broad and sustained anti-HBV immunity in most participants. When combined with antivirals, rapid and profound HBsAg declines were observed in all participants. This novel approach, in which anti-HBV immunity is sparked (ie, primed) with VRON-0200 and then fanned (ie, boosted) by HBV removal, could position VRON-0200 as a key agent for a future HBV functional cure.

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Research in Context

Evidence before this study

Durable clearance of circulating HBsAg and hepatitis B virus (HBV) DNA (ie, functional cure) after treatment is the optimal treatment goal for patients with chronic HBV infection. The major challenges to achieving this goal include the high HBV viral burden (high concentrations of HBV DNA and antigens) and the weakened innate and adaptive immune responses associated with chronic HBV infection. Newer and more potent investigational antiviral agents have shown progress with higher rates of end-of-treatment HBsAg loss. However, HBsAg rebound after treatment is frequent and rapid (eg, within 6 months) and necessitates re-initiation of HBV treatment in most patients, with functional cure observed primarily in patients with low baseline HBsAg concentrations. As a result, the addition of immune modulators, including the use of therapeutic vaccines, has been tried in an effort to improve responses. The addition of pegylated interferon modestly improves HBsAg declines, whereas therapeutic vaccines have shown little benefit when used alone or when combined with pegylated interferon or low doses of monoclonal anti-programmed cell death protein 1 antibodies; sustained loss of HBsAg concentration after treatment has been observed only in patients with baseline HBsAg concentrations of less than 1000 IU/mL. These modest benefits are offset by increased frequency and severity of treatment-related side-effects, some of which are serious and some having inconvenient and frequent dosing schedules. Finally, lead-in strategies with small interfering RNA or antisense oligonucleotides to first lower HBsAg concentrations before the addition of an immune modulator or a therapeutic vaccine have shown restricted benefits to date.
A formal literature search, clinical registry search, or meta-analysis was not conducted for this manuscript. The references included represent a targeted selection of peer-reviewed data gathered by the authors to support the clinical discussion and contextualise the evidence available before the study.

Added value of this study

This study was the first to evaluate VRON-0200, a first-in-class checkpoint modifier-containing immunotherapy, delivered by a therapeutic vaccine platform, in participants with chronic HBV infection receiving stable nucleos(t)ide therapy. A single VRON-0200 intramuscular prime dose was well tolerated and immunogenic and lowered HBsAg concentrations in most participants, despite VRON-0200 not directly targeting HBsAg. HBsAg declines were sustained and continued to decrease 360 days after a single dose, appeared independent of baseline HBsAg concentrations (<500 IU/mL), and continued to decline in 11 participants with long-term follow-up, with two achieving HBsAg loss within 2 years. The addition of an investigational antiviral regimen 28 days after a VRON-0200 prime dose produced rapid and profound HBsAg declines in all participants within 7 days, independent of their baseline HBsAg concentrations (<1000 IU/mL).

Implications of all the available evidence

VRON-0200 is the first new HBV immune modulator with durable anti-HBV activity since pegylated interferon and the first HBV therapeutic vaccine with documented anti-HBV clinical activity being observed in most treated patients. The broad and sustained anti-HBV immune restoration following VRON-0200 administration might help to prevent viral rebound upon antiviral discontinuation, reduce the need for HBV rescue medications, and improve overall functional cure rates. The administration of VRON-0200 before removing viral antigens appears key for combination functional cure treatments. HBsAg declines occurred independently of the baseline HBsAg concentrations, although the inclusion criteria were restricted to less than 500 IU/mL for VRON-0200 alone and less than 1000 IU/mL for the combination group. Thus, future VRON-0200-containing regimens might be expanded to include patient populations with higher baseline HBsAg concentrations. These data support the advancement of VRON-0200 into a larger phase 2b study evaluating functional cure, when VRON-0200 is given alone or with the addition of antiviral agents in patients with chronic HBV infection, as needed.

Introduction

Despite a preventive vaccine, chronic infection with hepatitis B virus (HBV) affects close to 257 million people, with an estimated 1·1 million deaths per year owing to liver-related complications.[1] Although nucleos(t)ide reverse transcriptase inhibitors effectively suppress DNA replication, they do not inhibit transcriptional activity of covalently closed circular DNA and integrated HBV DNA and hence cannot reduce the production of HBsAg.[2] The presence and persistence of HBsAg in the blood, especially at higher concentrations, are associated with increased risks for liver disease progression and hepatocellular carcinoma.[3] As a result, consensus panel guidelines and regulatory agencies recommend both undetectable HBV DNA and negative HBsAg findings at 6 months after withdrawal of finite treatment as the goal of therapy—referred to as functional cure.[4,5]

Chronic HBV infection is characterised by high concentrations of HBV antigen expression, impaired CD8+ T-cell functions, and a highly immunotolerant hepatic microenvironment, leading to peripheral tolerance and exhaustion of innate and adaptive host immune responses.[6] HBsAg plays a crucial role in this dysregulation, with high and persistent concentrations of antigen acting as an immune decoy.[7] The artificial removal of HBsAg with investigational antivirals such as antisense oligonucleotides or small interfering RNA (siRNA) inhibitors,[8–13] even to concentrations below the lower limit of detection (LLOD), appears to be inadequate to restore anti-HBV immunity, with high rates of HBsAg rebound and low overall rates of functional cures being observed after treatment discontinuation. Therefore, the addition of immune modulators is now considered necessary for a functional cure.[5] Investigational combination regimens that include pegylated interferon have improved functional cure rates; however, these improvements are modest and have predominantly been observed in patients with low baseline HBsAg concentrations (eg, <1000 IU/mL).[9,10,13,14] Vaccines aimed at stimulating CD8+ T-cell responses and restoring anti-HBV immunity, alone or in combination with investigational antivirals or monoclonal antibody-directed programmed cell death protein 1 checkpoint inhibitors (low-dose nivolumab), have largely been disappointing, with few patients experiencing HBsAg declines or functional cures, even after administering vaccines that directly target HBsAg.[15–17] These studies highlight the difficulties in restoring immune functions in patients with chronic HBV infection and the need for novel, easy-to-administer, well tolerated immunotherapies that can stimulate new and different immune responses.

VRON-0200, a first-in-class immunotherapy being developed for the functional cure of chronic HBV infection, comprises three components: (1) optimised HBV core and polymerase (pol), but not surface (S), antigens containing highly conserved sequences across genotypes A, B, C, and D; (2) a novel checkpoint modifier, herpes simplex virus-1 glycoprotein D (gD), into which the core and pol antigens are fused; and (3) a replicative defective chimpanzee adenoviral vector delivery platform of two distinct heterologous vectors, chimpanzee adenovirus serotype 7 (AdC7) and chimpanzee adenovirus serotype 6 (AdC6), to which the core–pol–gD gene sequence is inserted.[18] The check point modifier, gD, is an antagonist of the early B and T lymphocyte attenuator-CD160–herpes simplex virus entry mediator checkpoint, which acts to remove an early inhibitory signal of T-cell activation[19,20]—the overall effect is a lowering of the CD8+ T-cell activation threshold, which enhances responses to dominant epitopes and broadens T-cell activation to include subdominant epitopes,[18,21] which are generally not stimulated during a chronic infection.[22] This checkpoint modification occurs locally, at the injection site and regional draining lymph nodes, and between the antigen-presenting cell and CD8+ T-cell junction—this mechanism of action minimises the risk for serious off-target adverse events. VRON-0200, with checkpoint modification, is being investigated to enhance, broaden, and sustain anti-HBV immune responses, with the aim of preventing post-treatment viral rebound and improving functional cure response rates. This phase 1b clinical trial aimed to evaluate the safety, tolerability, immunogenicity, and antiviral efficacy of VRON-0200 in chronically HBV-infected patients receiving stable nucleos(t)ide antiviral therapy alone or in combination with investigational antivirals.

Methods

Study design and participants

This phase 1b, randomised, open-label, multicentre, dose-escalation, prime-only, and prime–boost vaccination trial was designed to evaluate the safety, tolerability, immunogenicity, and antiviral efficacy of two serologically distinct chimpanzee adenoviral vectors (AdC6 and AdC7) containing parts of HBV core and pol antigens fused to gD (VRON-0200), when administered alone and in combination with investigational antivirals to patients with chronic HBV infection. This was a multicentre trial performed at three centres across two countries: one site in Hong Kong and two sites in New Zealand.

A data monitoring committee reviewed the safety and progress of the study at various times. The protocol was reviewed and approved by an independent ethics committee at each investigational site before study initiation. Written informed consent was obtained from all participants before participation. The trial was conducted in compliance with the protocol; principles of the International Council for Harmonisation, Good Clinical Practice, and Declaration of Helsinki; and all applicable national regulations. Site personnel and sponsor staff oversaw the day-to-day conduct of the study. No major changes to the protocol were made during the study period. This study was registered at ClinicalTrials.gov (NCT06070051) and is active and fully enrolled.

Eligible participants were adults aged between 18 and 55 years with documented chronic HBV infection (defined as HBsAg positivity for >6 months with detectable HBsAg

at screening), who were HBeAg negative or positive, had normal liver function, showed no evidence of advanced liver fibrosis or cirrhosis, had HBsAg concentrations of less than 500 IU/mL (<1000 IU/mL in combination therapy groups receiving investigational antivirals), were receiving tenofovir (tenofovir alafenamide fumarate or tenofovir disoproxil fumarate) or entecavir, and had documented HBV viral load suppression (HBV DNA <40 IU/mL) for at least 12 months.

Randomisation and masking

Randomisation occurred per group in a 1:1 ratio for participants to be allocated to groups 1a or 1b, 2a or 2b, and 3a or 3b. This study was open-label, with participants, site personnel, and sponsor staff, being unblinded to the individual treatment assignments.

In group 1, participants were randomised to receive either a low prime dose (1×10^{10} viral particles [vp]) of AdC7 (group 1a) or AdC6 (group 1b) by intramuscular injection, with group 1a participants receiving an AdC6 boost at day 91 (figure 1). In group 2, participants were randomised to receive either a high prime dose (5×10^{10} vp) of AdC7 (group 2a) or AdC6 (group 2b) by intramuscular injection, with group 2a participants receiving an AdC6 boost at day 91. The use of each vector as a prime, at both a low and high vector dose, was implemented to evaluate if one vector or dose might induce improved immune responses or clinical activity, or both. The use of heterologous vectors of different serotype allow for optimal prime and boost evaluations because neutralising antibodies developed against one vector do not cross-react with the other. The study was amended to add a third group (group 3) in New Zealand, with participants randomised to receive a high-dose prime of AdC7 on day 1, followed by six monthly doses of an siRNA (elebsiran) plus an S-antigen-targeted monoclonal antibody (mAb; tobevibart), administered by subcutaneous injection, starting on day 28 alone (group 3b) or in combination with an AdC7 high-dose boost on day 196 (group 3a). All participants were assessed up to the end of study (up to day 360), and all participants remained on their nucleos(t)ide therapy throughout the study.

The study was amended to allow for long-term follow-up (LTFU) of participants in groups 1 and 2 who completed the study (day 360) and from the end of study visit up to 42 months after the first dose of VRON-0200. Data collected were part of the participant's regular clinical care during the LTFU, including markers of HBV infection such as HBsAg and hepatitis B surface antibody (HBsAb).

Procedures

Safety assessments included physical examination, measurement of vital signs, electrocardiography (12-lead electrocardiogram), and laboratory evaluations (serum chemistry, coagulation, haematology, and urinalysis tests), as well as a pregnancy test. These assessments were done at screening (up to 28 days before random assignment) and at various predefined timepoints throughout the study evaluation period. All adverse events were recorded from the time of consent to the final end of study visit (day 360) and included an assessment of intensity (mild, moderate, or severe), seriousness, and relationship to VRON-0200 (or siRNA and mAb for group 3) treatment. Participants were asked by the investigators at each study assessment if they had any adverse events or any changes in existing conditions. All adverse events and serious adverse events were coded using the Medical Dictionary for Regulatory Activities, and all adverse events and laboratory abnormalities were evaluated and documented using the US Food and Drug Administration guidance for industry.[23] Any adverse event that was not graded using the guidance was graded using Common Terminology Criteria for Adverse Events version 5.0.

Immunological assessments for changes in T-cell frequencies to interferon-γ (IFN-γ) responses to core and pol were performed via enzyme-linked immunosorbent spot assay (ELISpot; LLOD <30 spot-forming units per million peripheral blood mononuclear cells; background subtracted), with samples taken at two pretreatment timepoints within 28 days of prime dosing and for all groups at baseline (day 1) and days 14 and 28. When LLOD was reported, the absolute spot-forming unit values were used, if available. A positive immunological response was defined as two consecutive core plus pol ELISpot measurements at day 28 that were greater than the average of the two pretreatment timepoints and above the LLOD (referred to as a day 28 immunologic responder). To assess differences in IFN-γ responses in groups 1 and 2 among participants who received a VRON-0200 prime or prime and boost regimen, day 154 ELISpot responses were compared with pre-boost, day 91, responses in both dosing groups.

Virological assessments included measurements of HBV DNA, HBsAg, HBsAb, HBV pregenomic RNA (pgRNA), and hepatitis B core-related antigen concentrations in plasma at baseline and each study visit after dosing (HBV pregenomic RNA and hepatitis B core-related antigen analyses are ongoing and will be reported in a future publication).

Neutralising anti-vector antibody analyses were conducted in a sample of participants in groups 1 and 2 to evaluate both pre-anti-vector and post-anti-vector immunity, as assessed based on changes in neutralising antibody concentrations to the respective vector at three timepoints: day 1 (baseline, pre-dose), day 28, and day 91.

Screening assessments were conducted at local laboratories and then confirmed, when applicable, through central laboratories during the screening period and before randomisation and enrolment were initiated. Central laboratories were used for all routine biochemistry, haematology, serology, and virology evaluations, whereas speciality laboratories were used for IFN-γ ELISpot measurements (PPD Vaccine Sciences Lab, Richmond, VA, USA) and anti-vector neutralising antibody assessments (Rockland

Immunochemicals, Limerick, PA, USA). LTFU data were collected from local laboratories that were part of the patient's non-study care.

Outcomes

The primary endpoint for this study was to examine the safety and tolerability of two VRON-0200 vectors (AdC6 and AdC7), administered as a prime or prime–boost regimen alone or with elebsiran (VIR-2218) plus tobevibart (VIR-3434) in adult participants with chronic HBV infection receiving nucleos(t)ide therapy. The secondary endpoint was to assess quantitative changes in T-cell frequencies in blood from baseline, measured via IFN-γ ELISpot assay, after administering two VRON-0200 vectors, given as a prime or prime–boost regimen alone or in combination with elebsiran plus tobevibart.

Exploratory endpoints included the presence of pre-existing anti-vector antibodies and the development or persistence of anti-vector antibodies after VRON-0200 vaccination in groups 1 and 2 and the antiviral efficacy of VRON-0200 vectors alone or in combination with elebsiran plus tobevibart, as measured by change from baseline in plasma concentrations of HBV DNA, HBsAg, and HBsAb at various timepoints during the study.

Statistical analysis

No formal sample size was calculated in this phase 1b study because there was no formal statistical hypothesis testing. Study analyses are descriptive in nature. The sample sizes for each treatment group were typical for studies at this phase of development and were deemed sufficient to evaluate the study objectives. Data are presented as continuous variables, and frequencies and percentages are presented as categorical and ordinal variables. For IFN-γ ELISpot analyses, a one-sided paired t-test was used to compare responses from day 28 to pretreatment. Preclinical studies of gD, VRON-0200's checkpoint modifier, have shown hyperstimulation of T cells that can be impaired if a boost dose is administered too early (data on file, Virion Therapeutics)—as such, a two-sided paired t-test was used to evaluate responses from day 154 to day 91 in those participants who received a prime–boost regimen.

Role of the funding source

The funder of the study took the lead in designing, analysing, and interpreting the data and writing the manuscript in collaboration with the authors. The funder employed a contract research organisation to manage the study, which included data collection. The decision to submit this manuscript for publication was made by the funder and lead authors and was endorsed by all authors.

Results

Between Oct 26, 2023, and March 17, 2026, 35 participants were enrolled and dosed with VRON-0200 alone or in combination with investigational antivirals (figure 2); 34 participants completed the study (end of study, day 360),

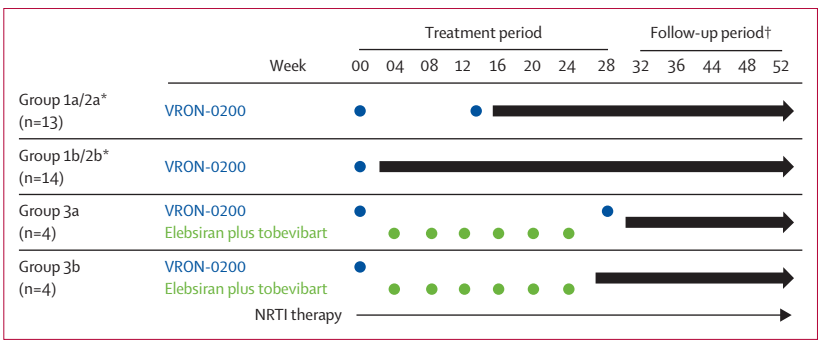

Figure 1: **Phase 1b study schema**

*Group 1: VRON-0200 intramuscularly 1×10^{10} vp; group 2: VRON-0200 intramuscularly 5×10^{10} vp. Group 1a and 2a: AdC7 prime followed by AdC6 boost at day 91 (week 13); group 1b and 2b: AdC6 prime only. Group 3a: AdC7 prime (5×10^{10} vp) followed by AdC7 boost at day 196 (5×10^{10} vp); group 3b: AdC7 prime only (5×10^{10} vp). †Week 52 (day 360) is the end of the study, which is the formal study period for VRON-0200. An elective long-term follow-up was implemented outside the core clinical schema to monitor ongoing anti-HBV activity, including sustained or continued HBsAg declines. Elebsiran is an siRNA, and tobevibart is an S-antigen targeted mAb. AdC7=chimpanzee adenovirus serotype 7. AdC6=chimpanzee adenovirus serotype 6. HBV=hepatitis B virus. mAb=monoclonal antibody. NRTI=nucleoside or nucleotide antiviral therapy. siRNA=small interfering RNA. vp=viral particles.

and one participant (group 3) withdrew consent on day 28 for personal reasons. All three groups were well matched (table 1). The participant median age was between 45 and 51 years; 28 (80%) of 35 participants were male, 30 (86%) were of Asian race, all, but one, were HBeAg negative (group 1b), and all were believed to have acquired HBV at birth. The median baseline HBsAg concentration was 179 IU/mL (range 16–623) for group 1, 149 IU/mL (10–563) for group 2, and 469 IU/mL (12–1169) for group 3. There were no observed differences in baseline demographics and characteristics between participants in groups 1a and 1b, 2a and 2b, or 3a and 3b.

At the end of the study, for all participants, VRON-0200 was well tolerated with no serious treatment-related adverse events (table 2). There were 89 reported adverse events in 24 participants, of which 38 were reported as treatment related: 34 were of grade 1 severity, with most symptoms consistent with those occurring after the intramuscular administration of an adenoviral vector (injection site pain, myalgias), and four grade 2 events of eczema, myalgia, headache, and injection site reactions that required topical treatment, aspirin, or paracetamol. All adverse events resolved without complications. No notable differences in the number or severity of adverse events were observed between participants who received AdC7 and AdC6 vectors, low and high vector doses, and prime alone and prime–boost treatments. Higher numbers of adverse events were reported for groups 2 and 3 than for group 1—in group 2, the higher numbers were related to four participants who had multiple, mild, reported adverse events (including one participant with nine separate events related to myalgia and influenza-like symptoms), and in group 3, the increased numbers were related to the additional monthly subcutaneous injections of elebsiran plus tobevibart. One participant in group 3 had Ramsay Hunt syndrome on day 79 (24 days after their second monthly dose of elebsiran plus tobevibart);

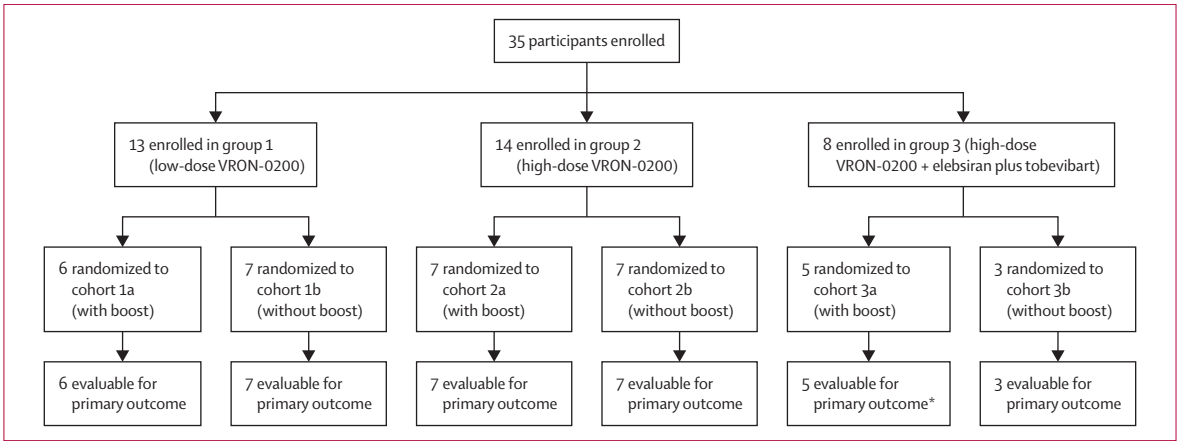

Figure 2: **Trial profile**
*One participant withdrew consent at day 28 and is included in demographics, baseline characteristics, and primary outcome (safety) analyses but is excluded from virological data analyses (no evaluable data).

	Group 1 (n=13)	Group 2 (n=14)	Group 3 (n=8)*
Age, years	45 (37–54)	46 (41–55)	51 (41–55)
Sex			
Male	12 (92%)	10 (71%)	6 (75%)
Female	1 (8%)	4 (29%)	2 (25%)
Race			
Asian	11 (85%)	13 (93%)	6 (75%)
Other	2 (15%)	1 (7%)	2 (25%)
BMI, kg/m^2	26·3 (20·2–32·0)	25·9 (19·7–31·6)	21·7 (18·6–32·0)
Chronic HBV infection	13 (100%)	14 (100%)	8 (100%)
Receiving nucleos(t)ide therapy >12 months	13 (100%)	14 (100%)	8 (100%)
Confirmed HBV DNA-suppressed >12 months	13 (100%)	14 (100%)	8 (100%)
Baseline HBsAg, IU/mL†	179 (16–623)	149 (10–563)	469 (12–1169)
Baseline HBsAg			
>500 IU/mL	1 (8%)	1 (7%)	4 (50%)
200 to <500 IU/mL	6 (46%)	4 (29%)	1 (13%)
100 to <200 IU/mL	2 (15%)	6 (43%)	0
<100 IU/mL	4 (31%)	3 (21%)	3 (38%)
Baseline ALT, × ULN			
1·5 to <2·0	1 (8%)	0	0
1·0 to <1·5	0	1 (7%)	0
<1·0	12 (92%)	13 (93%)	8 (100%)
HBeAg status at baseline			
Negative	12 (92%)	14 (100%)	8 (100%)

Data are median (range) or n (%). ALT=alanine aminotransferase. HBV=hepatitis B virus. ULN=upper limit of normal. *One participant withdrew consent at day 28 (group 3) and is included in demographic and baseline characteristics but excluded from clinical data (not evaluable). †As per protocol, participants had previous HBsAg concentration at screening <500 IU/mL in groups 1 and 2 and <1000 IU/mL in group 3.

Table 1: **Patient demographics and baseline characteristics**

clinical input from multiple medical disciplines evaluating the timing of the clinical presentation to dosing, the patient's medical history, and competing risk factors subsequently determined that this event was a non-treatment-related adverse event, and the patient received 5 days of high-dose steroid treatment. As a result, the next two monthly doses of elebsiran plus tobevibart were withheld (days 84 and 112) and subsequently re-initiated at the fifth scheduled monthly dose (day 140), as per protocol.

No treatment-related clinically significant laboratory abnormalities, including no elevations in alanine aminotransferase, aspartate aminotransferase, or bilirubin concentrations, were observed. In addition, no treatment-related study discontinuations were noted.

31 participants had IFN-γ ELISpot measurements available to both core and pol peptide pools at both pretreatment timepoints and days 14 and 28. Before vaccination, 23 (74%) of 31 participants had mean pretreatment ELISpot results below LLOD in both peptide pools. At day 28, after a single prime VRON-0200 dose, an overall 3·2-fold increase from baseline in core plus pol ELISpot values was observed (p=0·007; figure 3A). 11 participants (35%; groups 1 and 2, n=8; group 3, n=3) were day 28 immunologic responders, with an 8·3-fold increase from baseline at day 28 (figure 3B). Notably, increases in both core and pol peptide pools were observed on days 14 and 28. Day 28 immunologic responders received both AdC7 (n=7) and AdC6 (n=4) vectors and both low (n=7) and high (n=4) doses, and there were no notable differences in patient demographics or baseline characteristics of participants who had a positive day 28 ELISpot response.

21 participants in groups 1 and 2 had ELISpot measurements on days 91 and 154, including ten who received prime only and 11 who received prime followed by boost on day 91. Overall, ELISpot values at day 154 were 1·6-fold higher than those at day 91 (pre-boost), with no difference between participants who received a prime-only (p=0·17; one-sided paired t-test) or a prime–boost regimen (p=0·18; two-sided paired t-test; figure 3C).

In the anti-vector neutralising antibody evaluation, 36 samples were analysed from 12 participants. After VRON-0200 administration, neutralising antibody responses against either vector were observed in 11 (92%) of 12 participants, and these responses were sustained

	Group 1 (n=13)	Group 2 (n=14)	Group 3 (n=8)
Any AE	16	29	44
Grade 1	13	19	35
Grade 2	3	10	8
Grade 3 or 4	0	0	1*
SAE	0	0	1†
TRAE‡	5	16	17
Grade 1	4	14	16
Grade 2	1	2	1
Grade 3 or 4	0	0	0
AE leading to study discontinuation	0	0	0
Study discontinuation	0	0	1§
ALT elevations			
Grade 1	0	0	0
Grade 2	0	0	1*

Data are n. AE=adverse event. ALT=alanine aminotransferase. SAE=serious adverse event. TRAE=treatment-related adverse event. *Non-treatment related. †One participant in group 3 had a non-treatment-related adverse event that resulted in a two-dose elebsiran plus tobevibart treatment interruption. ‡No serious TRAEs, treatment-related discontinuations, or clinical laboratory abnormalities occurred; 38 TRAEs included four grade 2 events (eczema, myalgia, headache, and injection-site reaction), and all other TRAEs were grade 1. §Includes all 35 participants at the end of study (day 360), with the exception of one participant who withdrew consent at day 28 (end of study, day 28), which is a total of 12 268 patient safety days.

Table 2: **Safety and tolerability**

through day 91. Pre-existing vector immunity was identified in two participants; however, the administration of the prime VRON-0200 dose elicited anti-vector antibody responses in both the participants.

All participants underwent HBV DNA viral load and HBsAb testing at multiple timepoints. HBV DNA was undetectable in all participants at baseline and remained undetectable throughout the study. Baseline HBsAb was negative in all study participants and remained non-reactive (negative) in all participants of groups 1 and 2 throughout the study duration. All participants in group 3 seroconverted from HBsAb negative to positive within 7 days of the first investigational antiviral dose, and HBsAb positivity was maintained through the study cutoff date (a minimum of 3 months after the last dose of elebsiran and tobevibart).

Changes in HBsAg concentrations over time were assessed in all treatment groups. In groups 1 and 2, a single VRON-0200 prime dose resulted in HBsAg declines that were sustained or continued to decline in 23 (85%) of 27 participants till day 360 (figure 4A). HBsAg declines began around day 28 and were independent of baseline HBsAg concentrations—neither a VRON-0200 boost dose at day 91 nor IFN-γ ELISpot responses at day 28 (three day 28 immunologic responders did not have HBsAg declines at day 360) were observed to be associated with improved responses. 12 (52%) of the 23 participants with HBsAg declines at day 360 had a 50% or greater decline. No participant demographic or baseline characteristics were observed to be associated with HBsAg declines or the magnitude of the decline. 12 participants had LTFU up to 846 days after VRON-0200 prime dosing (figure 4B); 11 (92%) of 12 had continued declines, with HBsAg concentrations less than 10 IU/mL being reported in seven participants, less than 0·5 IU/mL in four, and less than 0·05 IU/mL (LLOD) in two, and six participants had declines of 1·4 $\log_{10}$ IU/mL or more from day 1.

In group 3, the administration of elebsiran and tobevibart at 28 days after a single VRON-0200 prime dose (group 3) resulted in rapid and profound HBsAg declines to less than 2 IU/mL within 7 days in all participants, regardless of baseline HBsAg concentrations (figure 5). Additional monthly doses resulted in continued declines. At day 196, 28 days after the last antiviral dose, all participants had HBsAg concentrations of 0·5 IU/mL or less (three of the seven elebsiran plus tobevibart treated participants were below LLOD), and, at day 259, 91 days (3 months) after their last antiviral dose, all but one participant (six [86%] of seven) had HBsAg concentrations that remained below 0·5 IU/mL, and one had HBsAg concentration that remained below LLOD.

Discussion

This is the first clinical study of VRON-0200, a novel immune modulator incorporating a checkpoint modifier of early T-cell activation. In patients with chronic HBV infection, VRON-0200 alone or in combination with investigational antivirals was safe and well tolerated, with no treatment-related serious adverse events or study discontinuations. Most adverse events were mild and consistent with an intramuscular dose of an adenoviral vector vaccine (injection site reactions and influenza-like symptoms).

In this trial, the participants had severely impaired anti-HBV immunity before VRON-0200 administration, with approximately three-quarters having IFN-γ ELISpot measurements below the level of detection in both core and pol peptide pools. A single VRON-0200 prime dose improved day 28 ELISpot responses by 3 fold from baseline; however, this improvement was predominantly driven by the 11 (35%) day 28 immunologic responders who had an 8-fold increase from baseline. A boost dose at day 91 did not improve ELISpot responses nor was being a day 28 immunologic responder associated with HBsAg declines at day 360. This study was not powered to evaluate T-cell changes and clinical responses. This absence of association between IFN-γ ELISpot measurements and HBsAg declines is consistent with previously reported findings[24] and might reflect the limitations of assessing IFN-γ in patients with severely impaired immune responses. Ongoing exploratory analyses are measuring other cytokines and biomarkers and their potential association with the clinical activity observed in this study.

A single VRON-0200 prime dose could induce broad anti-HBV immune activation and restoration, with durable HBsAg declines observed in most participants. HBsAg declines in patients with chronic HBV receiving stable nucleos(t)ide therapy occur at a rate of 0·007 $\log_{10}$ IU/mL

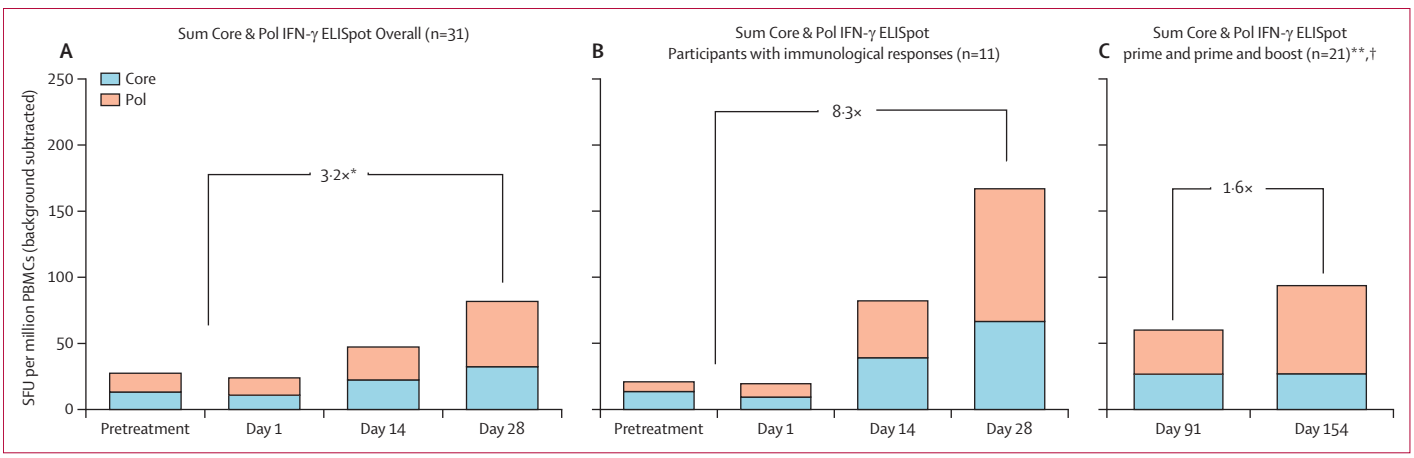

Figure 3: **IFN-γ ELISpot changes**

(A) Overall IFN-γ ELISpot changes (n=31) to HBV core and pol at day 28 following a single VRON-0200 intramuscular prime dose. *p=0·007 using one-sided paired t-test. (B) IFN-γ ELISpot changes to HBV core and pol at day 28 after a single VRON-0200 intramuscular prime dose in day 28 immunologic responders on day 28. (C) IFN-γ ELISpot changes (n=21) to HBV core and pol at day 154 versus day 91 after a single VRON-0200 intramuscular prime only or prime–boost (day 91) regimen. **Prime only, p=0·17 using one-sided paired t-test. †Prime and boost, p=0·18 using two-sided paired t-test. Core=HBV core. pol=HBV polymerase. HBV=hepatitis B virus. IFN-γ=interferon-γ. PBMC=peripheral blood mononuclear cell. SFU=spot-forming units.

per month (0·084 $\log_{10}$ IU/mL per year),[25] with an overall 10-year HBsAg loss incidence between 2% and 8%.[26,27] In this study, VRON-0200 was the only intervention given to groups 1 and 2, with all participants having stable HBsAg concentrations for the year before dosing; at day 360, after VRON-0200 prime dosing, 85% of participants had HBsAg declines, with over 50% experiencing HBsAg declines of more than 50% from baseline. Therefore, it is unlikely that natural HBsAg declines, independent of VRON-0200, were responsible for the observed responses. Checkpoint modification, via the inclusion of gD, enhances and broadens T-cell responses to include subdominant epitopes,[18,21] which are not normally stimulated during a chronic disease[22]—this novel mechanism of action might help to explain the observed clinical activity and is being investigated in ongoing exploratory studies. Additional exploratory studies, including those analysing T-cell antigen cross presentation, are investigating the mechanism by which VRON-0200 lowers HBsAg concentrations without directly targeting HBsAg. The absence of a boost dose improving HBsAg declines might be explained by the potency of a single VRON-0200 prime dose alone. A single dose stimulated early anti-HBV immune activation (day 28) and led to persistent anti-vector neutralising antibody titres till day 91, confirming effective drug uptake with robust and durable immune responses. As a result, a VRON-0200 boost dose is not required. Given that HBsAg declines were observed with both AdC7 and AdC6 vectors, at both low and high doses, with no differences in adverse events between vectors or vector doses, future studies will use a prime-only, high-dose (5×10^{10} vp) AdC7 vector-containing regimen.

Participants in groups 1 and 2 remained on their nucleos(t)ide therapy throughout the 360-day study period. Given that this study was a phase 1b trial, with safety and tolerability being the primary endpoint, no off-treatment assessments for functional cure were performed. However, the observed HBsAg declines occurred after a single VRON-0200 prime dose, meaning that the end of treatment was day 1. These sustained or continued HBsAg declines, up to 12 months after the end of treatment, and independent of baseline HBsAg concentrations, are different from what has been previously observed with HBV therapeutic vaccines.[15–17] For example, HBsAg declines after administering VTP-300 (ChAdOx1 prime, MVA boost) were infrequent, with three (17%) of 18 treated participants, all with baseline HBsAg concentrations less than 50 IU/mL, having a greater than 0·7 $\log_{10}$ decline 2 months after the end of treatment,[17] and no notable reductions in HBsAg concentrations were observed after vaccination with Brii-179 alone.[15] Although no participant in the VRON-0200 alone-treated groups had HBsAg loss through day 360, 11 (92%) of 12 participants evaluated in the LTFU had continued HBsAg declines, with seven participants having concentrations less than 10 IU/mL and two who achieved HBsAg loss. Under current treatment paradigms, these participants could be eligible for nucleos(t)ide discontinuation as part of their standard of care treatment. The timing and criteria for nucleos(t)ide discontinuation after anti-HBV immune restoration using an immune modulator such as VRON-0200 might be different from those when HBsAg loss, without immune restoration, occurs using antiviral treatment alone. For example, patients who achieve HBsAg loss while receiving pegylated interferon treatment had no additional benefits from continued antiviral therapy.[28] As such, there might not be any additional benefit to continuing nucleos(t)ide therapy once a patient's own immune response can control the virus, especially in HBeAg-negative patients with HBsAg loss or low HBsAg concentrations.[2] Additionally, from a global public health perspective, the benefits of maintained DNA suppression, in the presence of decreased or continuously declining HBsAg concentrations after a

A

Participant	Pretreatment	Day 1	Day 14	Day 28	Day 60	Day 91	Day 104	Day 118	Day 154	Day 360
1	493	622·9					NA			406·9
2*	496	562·6								39·8
3	380	441·9								297·7
4*	NA	389·9								105·9
5*	264	322·2								184·8
6	323	319·2								323·7
7	229	273·4								195·5
8*	234	271·0								127·9
9	224	265·4								271·2
10*	226	226·0								101·1
11	202	225·3								187·4
12	NA	221·8								197·9
13	170	178·9								141·2
14*	160	177·1								155·3
15*	132	150·2								67·0
16*	NA	148·3					NA			127·0
17	NA	124·8		NA					NA	22·0
18	102	112·8								81·9
19*	NA	103·1								47·0
20*	91	101·3								77·5
21	78	94·2								56·3
22	37	43·2								20·1
23	20	28·6								22·9
24*	24·5	25·4								0·7
25*	15	17·1								1·8†
26	15·5	16·4				NA				0·1
27*	8·3	9·7								3·8

Log_{10} change from baseline
≤–0·06
–0·07 to –0·49
–0·50 to –0·99
≥–1·00
Boost day 91
D28 IFN-γ "Responder"
*High dose

B

Participant	Within 1 year prior to enrolment	Phase 1b study through day 360		Long-term follow-up‡	
	HBsAg	Day 1 HBsAg	Day 360 HBsAg	HBsAg	Log_{10} IU/mL change from day 1
1	493	622·9	406·9	410	–0·18
2*	496	562·6	39·8	9·31	–1·78
4	NA	389·9	105·9	63·2	–0·79
7	229	273·4	195·5	138	–0·30
10	226	226	101·1	8·78	–1·41
14*	160	177·1	155·3	97	–0·26
21	78	94·2	56·3	46	–0·31
23	20	28·6	22·9	8·8	–0·51
24*	24·5	25·4	0·7	<0·05 (LLOD)	–2·71
25*	15·0	17·1	1·81†	0·32	–1·73
26	15·5	16·4	0·12	<0·05 (LLOD)	–2·52
27*	8·3	9·7	3·77	0·24	–1·60

Log_{10} change from day 1
≤–0·06
–0·07 to –0·49
–0·50 to –0·99
≥–1·00
Boost day 91
*High dose

Figure 4: **HBsAg declines over time for VRON-0200 alone (groups 1 and 2)**
(A) Heatmap of HBsAg changes over time in groups 1 and 2 (n=27; VRON-0200 intramuscular prime only or prime–boost (day 91) regimen). Day 28 immunologic responders are defined as having IFN-γ ELISpot measurements (sum of core plus pol) above the LLOD with two consecutive timepoint measurements greater than the average of the two pretreatment timepoints. Values at day 1 and day 360 are HBsAg concentrations (IU/mL). Participant 7 is HBeAg positive; all other participants are HBeAg negative. Pretreatment values are within 1 year before VRON-0200 dosing. (B) Heatmap of long-term follow-up HBsAg changes after end of study (VRON-0200 alone; groups 1 and 2; n=12). *High dose. †–0·97 log_{10} IU/mL decline. ‡Long-term follow-up day after VRON-0200 prime dose (in order from top to bottom participant): 838, 728, 541, 838, 664, 658, 846, 762, 593, 592, 738, and 619. IFN-γ=interferon-γ. LLOD=lower limit of detection. NA=not available. pol=HBV polymerase.

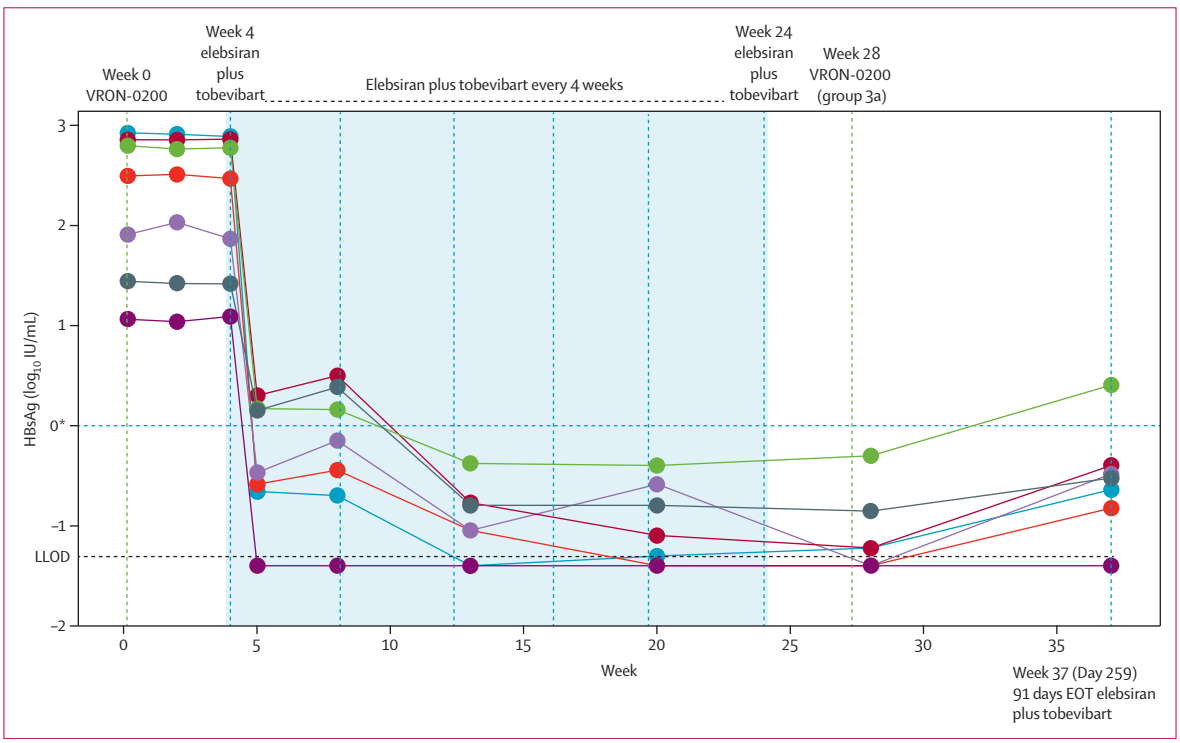

Figure 5: **HBsAg changes over time in group 3 (n=7; VRON-0200 intramuscular prime (day 1) in combination with 6 monthly doses of elebsiran plus tobevibart starting on day 28)**

The dotted line represents the LLOD. *HBsAg ≤1 IU/mL. EOT=end of treatment. LLOD=lower limit of detection.

single, well tolerated intramuscular dose of VRON-0200, cannot be overlooked. Six (22%) of the 27 VRON-0200-treated participants had baseline HBsAg concentrations of more than 100 IU/mL that declined to less than 100 IU/mL at day 360, a reduction that could potentially confer a 4-fold decrease in lifetime hepatocellular carcinoma risk.[29]

High concentrations of HBV antigens blunt host HBV-specific adaptive immune responses.[6,30] Studies evaluating the strategy of using a lead-in treatment with agents that remove HBsAg (siRNAs and antisense oligonucleotides), in an attempt to restore immune responses before administering an immune modulator or a therapeutic vaccine, however, have failed to improve functional cure rates.[14,31,32] 28 days after a VRON-0200 prime dose, the initiation of elebsiran plus tobevibart produced rapid and profound HBsAg declines in all seven patients within 7 days, independent of baseline HBsAg concentrations, and this decline continued with additional monthly antiviral doses. The speed and magnitude of the HBsAg declines with the addition of VRON-0200 were greater than previously observed with other investigational functional cure regimens, including elebsiran plus tobevibart, with or without pegylated interferon.[8,9,11,13,31] This observation suggests that pretreatment with VRON-0200 helps to reduce the duration of treatment needed to achieve a functional cure.

This new approach, termed Spark and Fan, is where anti-HBV immunity is sparked by a single dose of VRON-0200, priming the immune system to react to HBsAg, and then these responses are fanned (ie, boosted) by removing HBV with potent antivirals. Given that there are multiple agents and classes that effectively remove HBsAg, this approach might support VRON-0200 as a potential key backbone agent for many future HBV functional cure regimens. In addition, given that HBsAg declines were observed independent of baseline HBsAg in all three treatment groups (although the inclusion criteria were restricted to <500 IU/mL for VRON-0200 alone and <1000 IU/mL for the combination group), it is possible for VRON-0200 to expand patient populations to include higher baseline HBsAg concentrations. Lastly, other HBV-infected patient populations might benefit from VRON-0200's immune restoration and its lowering of HBsAg, including HIV–HBV and hepatitis D virus–HBV co-infection, metabolic dysfunction-associated steatohepatitis, and cirrhosis.

In summary, in this phase 1b study, VRON-0200 alone or in combination with elebsiran plus tobevibart was safe and well tolerated and had no observed safety concerns. Despite not targeting HBsAg, a single VRON-0200 dose could induce broad, sustained, or improving anti-HBV immunity in most participants till 360 days. In the 12 participants who had LTFU, 11 had continued HBsAg declines, with two achieving HBsAg loss. Additionally, when combined with antivirals, rapid and profound HBsAg declines were observed in all participants. The results of this phase 1b study support the use of a single, prime-only, high-dose, AdC7 vector-containing VRON-0200 regimen for future evaluation alone or with the addition of antiviral agents, as

needed, using the Spark and Fan strategy. A phase 2b trial assessing functional cure in chronically HBV-infected patients is in development.

Contributors

EG, GLHW, SC, AL, PM, MB, and THL were involved in conceptualising and designing the study. All authors were involved in the acquisition and interpretation of the data that were reported in the manuscript. AL and SC were responsible for writing the manuscript. All authors were involved in reviewing and revising the manuscript and had final responsibility for the decision to submit the publication.

Declaration of interests

EG has served as an adviser or speaker for AbbVie, Aligos Therapeutics, Ausperbio, Gilead Sciences, GlaxoSmithKline, and Tune Therapeutics. GLHW has served as an advisory committee member for AstraZeneca, Gilead Sciences, GlaxoSmithKline, and Janssen Pharmaceuticals and as a speaker for Abbott, AbbVie, Ascletis Pharma, Bristol-Myers Squibb, Echosens, Ferring Pharmaceuticals, Gilead Sciences, GlaxoSmithKline, Janssen Pharmaceuticals, Otsuka, and Roche and has received research grants from Gilead Sciences. SC, AL, and PM are executives at Virion Therapeutics, LLC, and own shares in the company. MB is an employee of PPD, part of Thermo Fisher Scientific, the laboratory contracted to perform work on this study. THL declares no competing interests.

Data sharing

Virion Therapeutics, LLC, shares anonymised individual patient data upon request or as required by law or regulation with qualified external researchers based on submitted curriculum vitae and reflecting non-conflict of interest. The request proposal must also include a statistician. Approval of such a request is at the sponsors' discretion and is dependent on the nature of the request, merit of the research proposed, availability of the data, and intended use of the data. Data requests should be sent to scurrie@viriontx.com.

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